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HIGH RESOLUTION NMR AND X-RAY CRYSTALLOGRAPHY DATA OF CAUDICIFOLIN FROM *EUPHORBIA ACAULIS*

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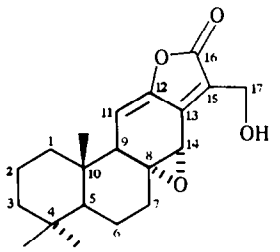
Key Word Index—*Euphorbia acaulis*; Euphorbiaceae; caudicifolin; ^{13}C NMR; X-ray crystallography.

Abstract—A diterpene lactone was isolated from the cold petrol (60–80°) extract of rhizomes of *Euphorbia acaulis*, a plant material used by a tribe of central India for curing various inflammatory disorder. The diterpene, which was observed to be identical to caudicifolin on the basis of its physical constants, was subjected to high resolution NMR spectroscopy and X-ray crystallography examination. This paper reports the salient features of the 2D ^{1}H NMR, ^{13}C NMR and X-ray crystallography data of the compound. ^{13}C NMR assignments were made by the use of proton noise decoupling, SFORD, APT and automatic spectral editing techniques. ^{1}H NMR assignments were made with the aid of a COSY experiment for long range couplings and NOE correlated 2D-experiments. The ^{1}H and ^{13}C NMR spectral assignments have been further corroborated by H/C correlation experimental results.

INTRODUCTION

A recent ethanobotanical survey [1] of the Tharu tribe of Kheri district of central India reported the use of a paste of rhizomes of *Euphorbia acaulis* for the cure of inflammatory disorders. Pharmacologists of our Institute have observed [2] that the residue from a cold petrol (60–80°) extract of the plant has a strong anti-inflammatory effect against induced oedema in experimental animals. Potency of the extract was observed to be equivalent to phenylbutazone, an established drug for the disorder.



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RESULTS AND DISCUSSION

The residue from the petrol (60–80°) extract, obtained by percolation of the powdered drug at 25°, was subjected to column chromatography over silica gel using petrol–ethyl acetate mixtures of increasing polarity as eluting solvent. Fractions obtained with petrol–ethyl acetate (95:5) gave a residue which was observed to be homogeneous on TLC. The material was obtained in a colourless crystalline form after repeated crystallizations from ethyl acetate and was coded as Ea-IV. Ea-IV, mp 184°, $[\alpha]_D^{19°}$ +97.1° (*c* 1%; $CHCl_3$), UV λ_{max}^{MeOH} nm: 285 (log ε 4.228); IR ν_{max}^{KBr} cm^{-1} 1740 (γ-lactone, α,β unsaturated C=O), 1650 (C=C) and 3550 (OH) was observed to very similar to caudicifolin (1) reported [3] earlier from *Euphorbia caudicifolia* (reported mp 177–182° and $[\alpha]_D$ +94.1°). The structure for caudicifolin was determined from the UV, IR and low resolution (60 MHz) ^{1}H NMR spectra and on the basis of assumed analogy to the established structure of jolkinolide reported earlier from *Euphorbia jolkine* [4], and which was also isolated from *Euphorbia caudicifolia* along with caudicifolin. In the ^{1}H NMR spectra (400 MHz) (Figs 1 and 2), the two protons on C-17 appeared as ABq (δ4.627, 4.667, *J* = 10 Hz). In addition a weak long range coupling involving six bonds between protons on C-17 and that on C-11 was observed in a COSY experiment optimized for long range coupling. H-11 was recorded at δ5.569 as a doublet (*J* = 5.5 Hz) due to its direct coupling with H-9. However, a doublet due to H-9 (δ2.642, *J* = 5.5 Hz) was observed to be a broadened one ($W_{1/2}$ = 1 Hz) due to its long range coupling with H-14 which was recorded as a doublet at δ4.005 (*J* = 1 Hz). Three tertiary methyl signals at δ0.723, 0.845 and 0.934 were assigned to three methyl groups at C-20, C-19 and C-18, respectively, on the basis of H/C correlation data (Fig. 3). The ^{13}C NMR assignments for this compound have been summarized in Fig. 4. The resolution of overlapping signals in ^{13}C NMR spectra was attained by use of APT, DEPT and spectral editing techniques. The assignments have been made by use of reported data for similar compounds, multiplicity of signals in SFORD spectra and use of heteroatom shielding and deshielding data. However, assignments for C-12 and C-13 are interchangeable.

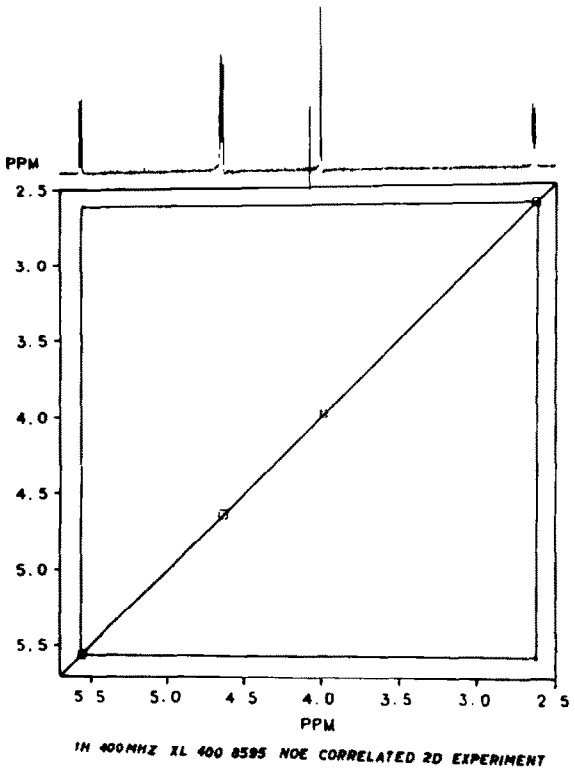


Fig. 1.

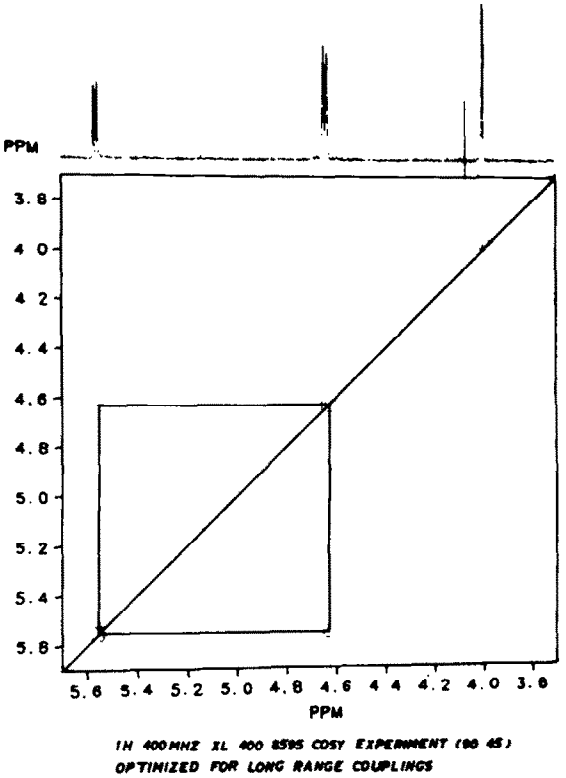


Fig. 2.

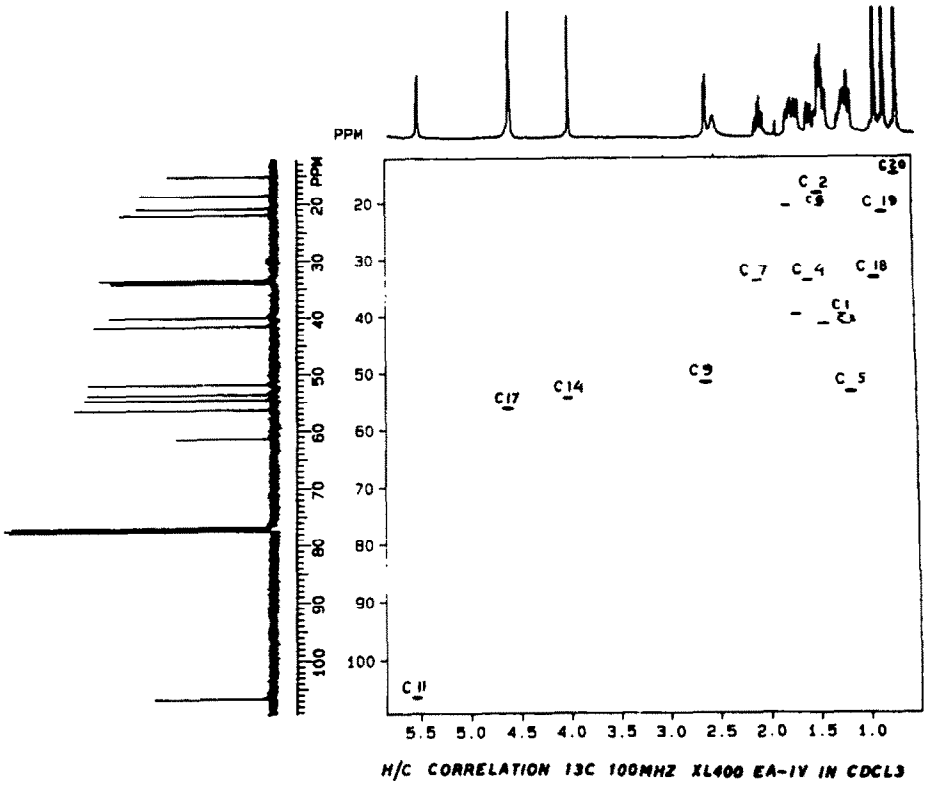
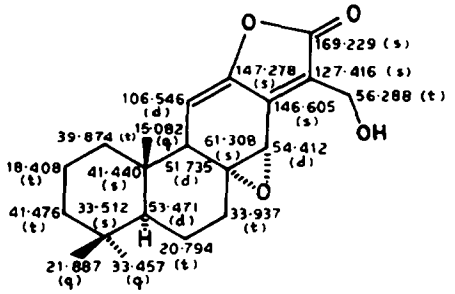


Fig. 3.



^{13}C-NMR IN $CDCl_3$, OBSERVED CARBON FREQUENCY 100·547 MHZ; REF. TMS; δ VALUES

Fig. 4.

X-ray crystallography

A single crystal of diterpene lactone Ea-IV, $C_{20}H_{26}O_4$ (M_r 330.428) obtained by recrystallization from ethyl acetate was subjected to X-ray analysis. The crystal data were as follows: approximate dimensions 0.20 × 0.25 × 0.30 mm; monoclinic; space group $P2_1$ ($Z = 2$); cell dimensions $a = 12.927(8)$, $b = 9.454(6)$, $c = 7.182(6)$ Å, $= 104.54(6)$, $v = 850(1)$ Å^3.

Lattice constants and diffracted intensities were derived from measurements carried out on a Rigaku AFC-5 FOS automated four-circle diffractometer equipped with a Cu rotor target. Intensity data were collected by 2θ-ω scanning with graphite-monochromated Cu-Kα radiation ($\lambda = 1.5418$ Å) within the limit of $2\theta < 120.0°$ to afford 1408 unique reflections. The phase problem was solved by the direct method (MULTAN) [5] and 11 plausible atomic positions were yielded on an E-map from 161 reflections with $E \geq 1.50$. Several cycles of isotropic least-squares and subsequent Fourier syntheses gave those of remaining non-hydrogen atoms. The hydrogen atoms except for that of the hydroxyl group were generated computationally on the basis of stereochemical and geometrical considerations. The structure was refined by block-diagonal least-squares (UNICS III) [6] to an R-value of 0.070 for the total reflections (all calculations were performed on a TOSBAC DS-600 computer). Anistotropic thermal parameters were used for non-hydrogen atoms, and isotropic thermal parameters for hydrogen atoms. Neither absorption correction now weighting scheme was employed.

An ORTEP [7] drawing of the structure is shown in Fig. 5 but the absolute configuration of the structure is undefined. The final atomic parameters, the bond lengths, bond angles and dihedral angles for non-hydrogen atoms are deposited at the Cambridge Crystallographic Data Centre.

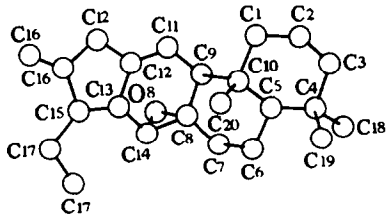


Fig. 5.

EXPERIMENTAL

^1H NMR spectra have been recorded on a Varian XL400 instrument with an acquisition time of 3.2 sec at a pulse width of 42° with 640 repititions. 64K FT size has been used for resolution enhancement. In the COSY experiment observed proton frequency was 399.843 MHz with spectral width and 2D-spectral width of 2129.9 Hz. Delay time used is 2.0 sec against acquisition time of 0.24 sec. First pulse of 90° against a pulse width of 45° has been employed. The number of repititions employed is 16 and number of increments is 256. Data processing has been done by pseudo echo shaped FT size IK × IK involving a total period of 2 hr 37 min.

In the COSY experiment for long range couplings first pulse of 90° has been used against a pulse width of 90°. Total time required for the operation is 2 hr 57.5 min. In NOE correlated 2D experiments, mixing time of 0.35 sec against delay time of 2.0 sec and acquisition time of 0.24 sec, has been employed. The number of repititions used was eight and number of increments employed was 512. Data processing took a total time of 3 hr 5.0 min. For the ^{13}C NMR spectra (recorded in Varian XL-400) the observed frequency was 100.547 MHz against an acquisition time of 1.501 sec at a pulse width of 45°. The number of repititions employed was 400. In H/C correlation experiments a spectral width of 9765.6 Hz against 2D-spectral width of 2133.6 Hz was employed. Pulse width of 90° against delay time of 1.5 sec and acquisition time of 0.105 sec has been used. Data processing for the experiment was done in pseudo echo shaped FT size 2K × 512 in a total time of 1 hr 51.9 min.

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